Previews

The Poststructural Festivities Begin

CIC chloride channels orchestrate the movement of chloride necessary for proper neuronal, muscular, cardiovascular, and epithelial function. In this issue of *Neuron*, Jentsch, Pusch, and colleagues use the structure of a bacterial CIC homolog to guide a mutagenic analysis of inhibitor binding to CIC-0, CIC-1, and CIC-2.

Most of us like to figure out how things work. We take things apart, we move them around, we *look* at them. It's hard to figure out how something works without looking at it or at least making a model. For ion channels, the classical arsenal of experimental approaches for inferring structure—electrophysiology coupled with mutagenesis and pharmacology—ultimately consists of indirect methods and blunt tools: they give us only a vague and incomplete look at the channel. Therefore, the field has been striving to see channels using crystallographic methods—a pursuit fueled by the discovery of bacterial homologs of neuronal ion channels—and that has been fulfilled for four channels and counting (Miller, 2000; Dutzler et al. 2002; Jiang et al., 2002). Structures, in turn, promise to focus the use and clarify the interpretation of the classical methods.

Structures resolve in an instant problems that have beset fields for years. Take, for example, the exquisite coordination of potassium ions by backbone carbonyl groups in the KcsA potassium channel or the tortuous transmembrane topology in the CIC chloride channels. But the best thing about a structure is its potential to direct functional questions previously inaccessible, and arguably nowhere was this more desperately needed than for the CIC family of chloride channels. Prior to the structure, the transmembrane topology, the nature of the pore, and even the number of pores per complex had been in controversy (Maduke et al., 2000). Now, with structure in hand, these issues are resolved, and we are freed to dig deep into the structure as a guide to detailed functional analysis. Indeed, complementing structure with function is not only useful but also necessary. For instance, most neurobiologists are interested primarily in the eukaryotic ion channels. However, all of the current structures represent bacterial homologs; with sequence identities in the 15% range, these proteins convey the basic folds of their eukaryotic counterparts but will surely differ in significant details. Furthermore, proteins are dynamic entities, yet crystallographic data are, by their nature, static. Full understanding will result from resourceful combination of structural and functional approaches.

Although the CIC channels don't make everyone's Top 10 list of important neuronal ion channels, surprising and important revelations are emerging (Jentsch et al., 2002). For example, defects in CIC-2 are responsible for certain common forms of idiopathic generalized epilepsy (Haug et al., 2003), and a mouse knockout of CIC-3 results in an unexplained and complete degeneration of the hippocampus (Stobrawa et al., 2001), so we have plenty of motivation for learning more about this ubiquitous family. Yet, at the molecular level, the CIC channels have been difficult to understand, largely because they defy conventional ion channel architecture. All of the well-known cation-selective ion channels share the same basic molecular design: they are oligomers containing a single pore along the axis of symmetry. In contrast, the CIC channels are two-pore homodimers, in which each pore is entirely formed from one subunit. Even a brief look at the structure of a CIC channel reveals the complexity dictated by this architecture: each subunit has 17 membrane-embedded α helices; very few of these actually span the entire membrane (hydropathy analysis was hopeless), and the chloride binding site is made from the coalescence of four different segments of sequence. The bottom line: in the absence of high-resolution structure, it was easier to predict Rube Goldberg machine designs than to interpret functional effects of channel mutagenesis.

In this issue of *Neuron*, the Jentsch and Pusch laboratories show how well structure can sharpen the blunt tool of mutagenesis (Estévez et al., 2003). They address the compelling question of the location of inhibitor binding in the mammalian channels CIC-1 and CIC-2. CIC-1, long known to be crucial for skeletal muscle activity, is inhibited by 9-anthracene carboxylic acid (9-AC), while CIC-2, strongly expressed in some GABA-ergic neurons and mutated in idiopathic generalized epilepsy, is unaffected by 9-AC. Starting with a chimeric approach, Estévez et al. closed in on an important region of the sequence. Next, they scrutinized this region, taking into consideration the sequence of CIC-0 from the *Torpedo* electric ray, which is not blocked by 9-AC, to narrow down to a sole serine residue largely responsible for the high affinity of 9-AC for CIC-1 versus CIC-2. (In the *Torpedo* channel, an additional mutation was needed to confer 9-AC sensitivity.) Similar, though not completely overlapping, results were found using another blocker, p-chloro-phenoxy-acetic acid (CPA). Without the structure of the bacterial homologs, this may well have been the end of the story. However, with a high-resolution model in hand, Estévez et al. were positioned to stage a directed mutagenesis attack that culminated by revealing a probable inhibitor binding site. The results are striking: residues that strongly affect inhibitor binding, while emanating from disparate segments of sequence, cluster to form a hydrophobic pocket that is partially exposed to the intracellular side of the channel.

This binding pocket abuts some of the chloride-coordinating side chains and backbone atoms that line the channel pore. Interestingly, mutations at the chloride-coordinating positions did not necessarily affect pore properties: Y578, a highly conserved residue whose hydroxyl group coordinates a Cl^- ion seen in the prokaryotic CIC crystal structure, can be mutated to alanine or tryptophan with no measurable effect on either single-channel conductance or selectivity. This surprising re-
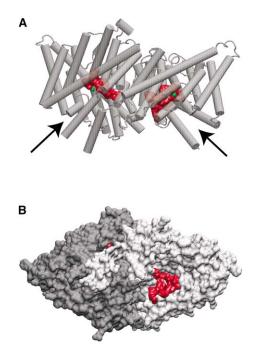
Figure 1. Structure of the *Salmonella* ClC Chloride Channel Showing Residues Corresponding to Those in ClC-1 that Have "High-Impact" Effects on 9-AC Binding

(A) Side view, tilted slightly to reveal the bound Cl⁻ (green). Residues with high impact on 9-AC binding are red. The predicted Cl⁻-access pathway in each subunit is indicated with an arrow.

(B) Solvent-accessible surface rendition of the channel viewed from the intracellular side of the membrane. In the right-hand subunit (light gray), three helices (A, D, and R) have been removed in order to reveal the high-impact residues (red). In the left-hand subunit (dark gray), a glimpse of red suggests one potential access pathway to the inhibitor binding site.

Protein Data Bank accession number is 1KPL (Dutzler et al., 2002). Images generated with VMD (Humphrey et al., 1996).

sult is perhaps understandable in light of the recently published structure of the *E. coli* ClC at increased resolution, which reveals two additional Cl⁻ binding sites, one extracellular and one intracellular to the "Y578" site (Dutzler et al., 2003).

But other questions remain. How does the inhibitor get in there? The authors suggest, based on the effects on inhibitor binding kinetics of the mutation K485E (a residue known to line the conduction pathway), that 9-AC may reach the binding site via the chloride-selective permeation pathway. However, they also point out that the results do not exclude an alternate access pathway. The uncertainty arises because when the results are transposed onto the bacterial channel structure, the binding site can be only vaguely glimpsed from the intracellular face of the channel, and no access pathway is physically big enough to accommodate a 9-AC molecule (see Figure 1). Two possibilities present themselves: either the intracellular face of ClC-0, -1, and -2 is quite different from that of the prokaryotic channels, or significant movements occur involving the intracellular portion of the proteins as they open and close. This is a question for future experiments, which are also inspired by another newly published crystal structure, of an *E. coli* ClC containing a mutation (E148Q) that in ClC-0 causes the

channels to be constitutively open (Dutzler et al., 2003). The sole difference observed between the mutant ("open") and wild-type ("closed") channels is a swinging movement of the *extracellular* side chain of residue 148. If this is indeed the only movement that occurs upon channel opening, then ClC-0, -1, and -2 must harbor more space in their internal vestibules than does the *E. coli* homolog. On the other hand, it is possible that another conformational change is required for channel opening, as has been proposed for ClC-0 (Chen and Miller, 1996) and is implied by the observation that an inhibitor closely related to CPA has different affinities for open versus closed channels (Pusch et al., 2002).

The current study by Estévez et al., together with the structures, stimulates many other questions. Can the inhibitor binding pocket be reiterated in any of the other ClCs? Although the other eukaryotic CLCs, like the bacterial channels, are only ∼15%–20% identical to ClC-0, -1, and -2, three of the four segments of protein that are "high impact" for inhibitor binding are highly conserved. Are the bacterial channels blocked by any of the known ClC blockers or by any natural inhibitors? Will it be possible to use homology models and molecular docking to discover (sorely needed) specific inhibitors of the various ClC family members (Shoichet et al., 2002)? Out of the scope of this preview are the many questions regarding molecular mechanisms and regulation of these channels. Estévez et al. have initiated the long-awaited ultimate structure-function paradigm for studying ClC chloride channels, and no doubt such an approach will bear much fruit in the coming years. Now that we can look at the channels, we get to start taking them apart.

Merritt Maduke[1] and Joseph A. Mindell[2]
[1]Stanford University
Department of Molecular and Cellular Physiology
B155 Beckman Center, 279 Campus Drive
Stanford, California 94305
[2]Membrane Transport Biophysics Unit
NIH/NINDS
36 Convent Drive, Bldg. 36, Room 1C25, MSC 4066
Bethesda, Maryland 20892

Selected Reading

Chen, T.Y., and Miller, C. (1996). J. Gen. Physiol. *108*, 237–250.

Dutzler, R., Campbell, E.B., Cadene, M., Chait, B.T., and MacKinnon, R. (2002). Nature *415*, 287–294.

Dutzler, R., Campbell, E.B., and MacKinnon, R. (2003). Science, in press. Published online March 20, 2003. 10.1126/science.1082708

Estévez, R., Schroeder, B.C., Accardi, A., Jentsch, T.J., and Pusch, M. (2003). Neuron *38*, this issue, 47–59.

Haug, K., Warnstedt, M., Alekov, A.K., Sander, R., Ramírez, A., Poser, B., Maljevic, S., Hebeisen, S., Kubisch, C., Rebstock, J., et al. (2003). Nat. Genet., in press. Published online March 3, 2003. 10.1038/ng1121.

Humphrey, W., Dalke, A., and Schulten, K. (1996). J. Mol. Graph. *14*, 33–38.

Jentsch, T.J., Stein, V., Weinreich, F., and Zdebik, A.A. (2002). Physiol. Rev. *82*, 503–568.

Jiang, Y., Lee, A., Chen, J., Cadene, M., Chait, B.T., and MacKinnon, R. (2002). Nature *417*, 523–526.

Maduke, M., Miller, C., and Mindell, J.A. (2000). Annu. Rev. Biophys. Biomol. Struct. *29*, 411–438.

Miller, C. (2000). Neuron *25*, 7–9.

Pusch, M., Accardi, A., Liantonio, A., Guida, P., Traverso, S., Conte Camerino, D., and Conti, F. (2002). Mol. Membr. Biol. *19*, 285–292.

Shoichet, B.K., McGovern, S.L., Wei, B., and Irwin, J.J. (2002). Curr. Opin. Chem. Biol. *6*, 439–446.

Stobrawa, S.M., Breiderhoff, T., Takamori, S., Engel, D., Schweizer, M., Zdebik, A.A., Bosl, M.R., Ruether, K., Jahn, H., Draguhn, A., et al. (2001). Neuron *29*, 185–196.

Motor "Binding:" Do Functional Assemblies in Primary Motor Cortex Have a Role?

In this issue of *Neuron*, Jackson and colleagues describe a functional correlate of neural synchrony related to movement control. Synchrony strength in cortico-motoneuronal output neurons in primary motor cortex depended upon similarity of these neurons' connectivity pattern with the spinal cord. These results could form the foundation for subsequent investigations of motor binding.

Neural synchrony has been observed throughout the CNS, particularly in sensory systems, and theories have abounded concerning its functional role. Preeminent among them is the theory that synchrony is the basis for perceptual binding (von der Malsburg and Schneider, 1986; Eckhorn et al., 1988). Since debate swirls about the meaning of neural synchrony in certain sensory processing areas (e.g., see Bair et al., 2001; Thiele and Stoner, 2003), a definitive role for neural synchrony in functional brain organization necessarily remains elusive. In this issue of *Neuron*, Jackson and colleagues (Jackson et al., 2003) explore the synchrony problem within the context of brain control of voluntary movement. Not unlike processing needed to form coherent percepts, the brain systems mediating voluntary actions must aggregate disparate spiking patterns to form spatially and temporally coherent neural codes that then drive α motor neurons and their associated muscles. Essentially, motor binding seems exactly what motor structures of the mammalian brain do—high-level coordination of simple and complex voluntary movements.

Movement control and the primary motor cortex (M1) of primates have particularly useful features in determining the functional significance of neural synchrony and its potential role for motor binding. Movement provides an extensive repertoire of experimental variables to manipulate and to assess from input to output streams. In contrast, richness in perceptual binding work focuses fundamentally on input manipulations and a single, binary response variable: perceived or not perceived. M1 seems a model site to investigate functional significance of synchrony. Preeminent among neocortical areas, M1 is ideally situated as the collector of multifarious, highly processed integrated inputs that then become relayed to the brain stem and spinal cord to express neural computations related to sensation, perception, and thought for voluntary action. Functional and anatomical features of M1 make it a particularly likely neocortical candidate site for implementation of motor binding. The intrinsic organization of M1 has distributed and overlapping movement representations, suggestive of intrinsic substrates for coordination (Sanes and Schieber, 2001). Additionally, M1 has profuse intracortical horizontal connections that exhibit short-term and long-term plasticity (Sanes and Donoghue, 2000), a feature likely needed for a structure devoted to synthesizing information to yield highly complex output.

Jackson et al. (2003) focused their work on a special type of neuron in M1: the cortico-motoneuronal (CM) cell (see Porter and Lemon, 1993, for a comprehensive review of CM cells). Anatomical and functional properties of the CM cell contribute to its use as a model for understanding neuromotor control and for investigating neural synchrony. CM cells have monosynaptic excitatory contacts on multiple α motor neurons in a single motor neuron pool, and via interneurons exert inhibitory effects on other, antagonist motor neurons. The synaptic effects of direct M1 input onto α motor neurons have been well characterized. Axons of individual CM cells also branch to contact neurons in other motor neuron pools; their aggregate branching pattern yields a muscle field that describes the functional coupling, both facilitatory and suppressive, of a CM cell to a set of muscles. Commonly, a muscle field for a CM cell comprises motor neuron pools having synergistic actions, potentially representing distal and proximal voluntary movements. While CM cells constitute a small proportion of all layer V cells—approximately 10%–20% of pyramidal tract neurons (PTN) in M1—their known anatomic and functional properties can be exploited to reveal general neural processing properties. A further advantage of studying neural synchrony of CM cells is that it can be done in awake preparations.

Synchrony in M1 was first observed nearly 20 years ago (see Jackson et al., 2003, for a review of relevant work). More recently, neural synchrony in M1 has been related to movement direction coding and specific aspects of task performance. However, these studies have not placed neural synchrony into the larger context of motor binding. New findings from Jackson et al. (2003) and Hatsopoulos et al. (2003) bring the connection between motor binding and M1 neural synchrony substantially forward.

Jackson et al. took advantage of the muscle field feature of the CM system to investigate the relationship between M1 neural synchrony and CM output. The spiking of neurons was recorded with a multiple-electrode array in a zone of M1 expected to yield functional relationships to muscles related to hand function; this sample was further restricted to CM cells. The spike triggered averaging (STA) method was used to characterize the muscle field of each CM cell. As commonly employed in neuromotor control, STA implemented between spiking and the electromyogram (EMG) can reveal both post-spike facilitation from CM cell action, a short-latency and transient increase in the EMG, or post-spike suppression, a transient decrease in the EMG. As expected, STA revealed the functional connection of CM cells to